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FILE 'HOME' ENTERED AT 11:31:01 ON 25 SEP 1998

=> fil caplus

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.30	0.30

FILE 'CAPLUS' ENTERED AT 11:32:06 ON 25 SEP 1998
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FILE COVERS 1967 - 25 Sep 1998  VOL 129 ISS 13
FILE LAST UPDATED: 25 [redacted] 1998  (980925/ED)

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.

  This file supports REG1stRY for direct browsing and searching of
  all substance data from the REGISTRY file.  Enter HELP FIRST for
  more information.

=> s amylase and active

         27771 AMYLASE
          4695 AMYLASES
         28456 AMYLASE
                 (AMYLASE OR AMYLASES)
        497143 ACTIVE
           204 ACTIVES
        497269 ACTIVE
                 (ACTIVE OR ACTIVES)
L1        1774 AMYLASE AND ACTIVE

=> s amylase (10a) active

         27771 AMYLASE
          4695 AMYLASES
         28456 AMYLASE
                 (AMYLASE OR AMYLASES)
        497143 ACTIVE
           204 ACTIVES
        497269 ACTIVE
                 (ACTIVE OR ACTIVES)
L2         619 AMYLASE (10A) ACTIVE

=> s amylase (10a) activ? (5a)residue

         27771 AMYLASE
          4695 AMYLASES
         28456 AMYLASE
                 (AMYLASE OR AMYLASES)
       2249537 ACTIV?
        168731 RESIDUE
        212567 RESIDUES
        329369 RESIDUE
                 (RESIDUE OR RESIDUES)
L3          47 AMYLASE (10A) ACTIV? (5A)RESIDUE

=> s l3 (10a)bacillus

         47106 BACILLUS
          3770 BACILLI
           136 BACILLIS
         49903 BACILLUS
                 (BACILLUS OR BACILLUSES OR BACIL)
L4           3 L3 (10A)BACILLUS

=> d1-

D1- IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> d 1-
```

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 3 CAPLUS COPYRIGHT 1998 ACS
AN 1993:97075 CAPLUS
DN 118:97075
TI Functional roles of **active** site **residues** of **Bacillus** polymyxa .beta.-**amylase**
AU Uozumi, Nobuyuki
CS Fac. Eng., Nagoya Univ., Nagoya, 464-01, Japan
SO Ann. N. Y. Acad. Sci. (1992), 672(Enzyme Engineering XI), 24-8
CODEN: ANYAA9; ISSN: 0077-8923
DT Journal
LA English

L4 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1998 ACS
AN 1992:250998 CAPLUS
DN 116:250998
TI Site-directed mutagenesis of **active** site **residues** in **Bacillus** subtilis .alpha.-**amylase**
AU Takase, Kenji; Matsumoto, Takashi; Mizuno, Hiroshi; Yamane, Kunio
CS Dep. Mol. Biol., Natl. Inst. Agrobiol. Resourc., Tsukuba, Japan
SO Biochim. Biophys. Acta (1992), 1120(3), 281-8
CODEN: BBACAQ; ISSN: 0006-3002
DT Journal
LA English

L4 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1998 ACS
AN 1985:127861 CAPLUS
DN 102:127861
TI An **active** center tryptophan **residue** in liquefying .alpha.-**amylase** from **Bacillus** amyloliquefaciens
AU Kochhar, Sunil; Dua, Ramji D.
CS Biochem. Lab., Indian Inst. Technol., New Delhi, 110016, India
SO Biochem. Biophys. Res. Commun. (1985), 126(2), 966-73
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English

=> d 1- ab

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 3 CAPLUS COPYRIGHT 1998 ACS
AB The effects of mutation of Cys, His, and Glu residues in conserved regions of .beta.-amylase on the catalytic properties of the enzyme are discussed.

L4 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1998 ACS
AB Site-directed mutagenesis of B. subtilis N7 .alpha.-amylase was performed to evaluate the roles of the active site residues in catalysis and to prep. an inactive catalytic-site mutant that could form a stable complex with natural substrates. Mutation of Asp-176, Glu-208, and Asp-269 to their amide forms resulted in a >15,000-fold redn. is its specific activity, but all of the mutants retained considerable substrate-binding abilities as estd. by gel electrophoresis in the presence of sol. starch. Conversion of His-180 to Asn resulted in a 20-fold redn. in kcat with a 5-fold increase in km for a maltopentaose deriv. The relative affinities for acarbose vs. maltopentaose were also compared between the mutants and wild-type enzyme. The results were consistent with the roles previously proposed in Taka-amylase A and porcine pancreatic .alpha.-amylase based on their x-ray crystallog. anal., although

different pairs had been assigned as catalytic residues for each enzyme. Anal. of the residual activity of the catalytic-site mutants by gel electrophoresis suggested that it derived from the wild-type enzyme contaminating the mutant prepns., which could be removed by use of an acarbose affinity column; thus, these mutants were completely devoid of activity. The affinity-purified mutant proteins should be useful for elucidating the complete picture of the interaction of this enzyme with starch.

L4 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1998 ACS
AB Liquefying .alpha.-amylase from B. amyloliquefaciens was inactivated by N-bromosuccinimide. Preincubation of the enzyme with either substrate or competitive inhibitor provided significant protection against inactivation. The relation between activity loss and the no. of tryptophan residues modified, as well as presence of substrate or inhibitor in the reaction mixt., demonstrated that only 1 of 3 modifiable tryptophan residues was at or near the active center. The apparent Km of the modified enzyme for sol. starch increased substantially, thus implicating the sensitive tryptophan residue in the substrate binding region of the enzyme.

```
=> d 1- ab

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):n


=> dis his


     (FILE 'HOME' ENTERED AT 11:31:01 ON 25 SEP 1998)

     FILE 'CAPLUS' ENTERED AT 11:32:06 ON 25 SEP 1998
L1          1774 S AMYLASE AND ACTIVE
L2           619 S AMYLASE (10A) ACTIVE
L3            47 S AMYLASE (10A) ACTIV? (5A)RESIDUE
L4             3 S L3 (10A)BACILLUS

=> amylase (15a)active center

AMYLASE IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s amylase (15a)active center

         27771 AMYLASE
          4695 AMYLASES
         28456 AMYLASE
                 (AMYLASE OR AMYLASES)
        497143 ACTIVE
           204 ACTIVES
        497269 ACTIVE
                 (ACTIVE OR ACTIVES)
        161103 CENTER
         93225 CENTERS
        225957 CENTER
                 (CENTER OR CENTERS)
          9811 ACTIVE CENTER
                 (ACTIVE(W)CENTER)
L5          30 AMYLASE (15A)ACTIVE CENTER

=> s l5 (15a)bacillus

         47106 BACILLUS
```

```
          3770 BACILLI
           136 BACILLI
         49903 BACILLUS
                (BACILLUS OR BACILLUSES OR BACIL)
L6           4 L5 (15A)BACILLUS

=> d 1-

YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):n


=> d l6 not l4

L4 IS NOT VALID HERE
For an explanation, enter "HELP DISPLAY".

=> s l6 not l4

L7           3 L6 NOT L4

=> d 1-

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y


L7   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 1998 ACS
AN   1995:68546  CAPLUS
DN   122:26628
TI   Properties and active center of the thermostable branching enzyme
     from Bacillus stearothermophilus
AU   Takata, Hiroki; Takaha, Takeshi; Kuriki, Takashi; Okada, Shigetaka;
     Takagi, Masahiro; Imanaka, Tadayuki
CS   Biochemical Research Laboratories, Ezaki Glico Co., Ltd., Osaka,
     555, Japan
SO   Appl. Environ. Microbiol. (1994), 60(9), 3096-104
     CODEN: AEMIDF; ISSN: 0099-2240
DT   Journal
LA   English

L7   ANSWER 2 OF 3  CAPLUS  COPYRIGHT 1998 ACS
AN   1989:547515  CAPLUS
DN   111:147515
TI   Functional improvement of enzymes by recombinant DNA technology
AU   Yamane, Kunio
CS   Inst. Biol., Univ. Tsukuba, Tsukuba, 305, Japan
SO   Gekkan Fudo Kemikaru (1989), 5(7), 31-7
     CODEN: GFKEEX; ISSN: 0911-2286
DT   Journal; General Review
LA   Japanese

L7   ANSWER 3 OF 3  CAPLUS  COPYRIGHT 1998 ACS
AN   1985:434160  CAPLUS
DN   103:34160
TI   Chemical modification of liquefying .alpha.-amylase:  role of
     tyrosine residues at its active center
AU   Kochhar, Sunil; Dua, Ramji D.
CS   Biochem. Lab., Indian Inst. Technol., New Delhi, 110016, India
SO   Arch. Biochem. Biophys. (1985), 240(2), 757-67
     CODEN: ABBIA4; ISSN: 0003-9861
DT   Journal
LA   English

=> d 1- ab

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y
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L7 ANSWER 1 OF 3 CAPLUS COPYRIGHT 1998 ACS
AB Although the branching enzyme (EC 2.4.1.18) is a member of the .alpha.-amylase family, the characteristics are not understood. The thermostable branching enzyme gene from Bacillus stearothermophilus TRBE14 was cloned and expressed in Escherichia coli. The branching enzyme was purified to homogeneity, and various enzymic properties were analyzed by the author's improved assay method. About 80% of activity was retained when the enzyme was heated at 60.degree.C for 30 min, and the optimum temp. for activity was around 50.degree.C. The enzyme was stable in the range of pH 7.5 to 9.5, and the optimum pH was 7.5. The nucleotide sequence of the gene was detd., and the active center of the enzyme was analyzed by means of site-directed mutagenesis. The catalytic residues were tentatively identified as two Asp residues and a Glu residue by comparison of the amino acid sequences of various branching enzymes from different sources and enzymes of the .alpha.-amylase family. When the Asp residues and Glu were replaced by Asn and Gln, resp., the branching enzyme activities disappeared. The results suggested that these three residues are the catalytic residues and that the catalytic mechanism of the branching enzyme is identical to that of .alpha.-amylase. On the basis of these results, four conserved regions including catalytic residues and most of the substrate-binding residues of various branching enzymes are proposed.

L7 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1998 ACS
AB A review, with 15 refs., on the detn. of the **active center** and elevation of thermostability of **Bacillus** .alpha.-**amylase** by site-directed mutagenesis; the possibility of starch prodn. by cyclomaltodextrin gluconotransferase as a result of introducing a mutation at the active site of cyclization; and on the improvement of subtilisin activity at room temp.

L7 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1998 ACS
AB Liquefying .alpha.-amylase from Bacillus amyloliquefaciens was inactivated by treatment with tetranitromethane and N-acetylimidazole. The loss of activity occurred with modification of 5 tyrosine residues. Preincubation of the enzyme with either the substrate or the competitive inhibitor at satg. levels provided complete protection against inactivation. However, the presence of substrate/inhibitor in the reaction mixt. protected only 2 of the 5 modifiable tyrosine residues, suggesting the involvement of only 2 tyrosine residues at the active center. This was confirmed when hydroxylamine treatment of the acetylated enzyme fully restored the enzymic activity. Both nitration and acetylation increased the apparent Km of the enzyme for sol. starch, which indicated that the tyrosine residues are involved in substrate binding. Redn. of nitrotyrosine residues to aminotyrosine residues failed to restore the enzymic activity. Thus, the loss of activity on modification of tyrosine residues was ascribed to conformational perturbations, and not simply to changes in the ionic character of tyrosine residues.

=> dis his

(FILE 'HOME' ENTERED AT 11:31:01 ON 25 SEP 1998)

FILE 'CAPLUS' ENTERED AT 11:32:06 ON 25 SEP 1998
L1 1774 S AMYLASE AND ACTIVE
L2 619 S AMYLASE (10A) ACTIVE
L3 47 S AMYLASE (10A) ACTIV? (5A)RESIDUE
L4 3 S L3 (10A)BACILLUS
L5 30 S AMYLASE (15A)ACTIVE CENTER

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L6              4 S L5 (15A)BACILLUS
L7              3 S L6 N[redacted]L4

=> log h

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    31.27      31.57

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -3.09      -3.09

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